REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2003	Annual (1 Jul 2002 - 30 Jun 2003)

4. TITLE AND SUBTITLE
Genetic Analysis of a Single Nucleotide Polymorphism in the Matrix Metalloproteinase 1 Promoter in Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0221

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Tumor invasion requires destruction of collagen, and is accomplished by matrix metalloproteinase-1 (MMP-1). A single nucleotide polymorphism (SNP) in the MMP-1 promoter enhances transcription of this gene. The SNP is at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a binding site (5 -AGGA-3) for the Ets transcription factors. Allele frequency is: 25% =1 G homozygous, 25% =2 G homozygous, and 50% = 1G/2G heterozygous. We hypothesized that the 2G SNP was associated with aggressive breast cancer. MMP-1 is on chromosome 11q22.2-22.3, a region associated with Loss of Heterozygosity (LOH) in breast cancer, and that retaining the 2G allele after LOH provided tumors with an advantage for progression. Therefore, we (1) genotyped DNA from normal tissues and metastatic breast tumors for the SNP (2) evaluated tumors for LOH, and (3) measured MMP-1 mRNA levels. Of the 58 individuals genotyped, 24 were heterozygous, and of these only 5 underwent LOH, with 3 retaining the 2G allele and 2 retaining the 1G allele. mRNA analysis of tissues from 45 breast cancer patients revealed substantial MMP-1 mRNA expression in 32. Thus, although LOH may not favor the 2G allele, MMP-1 mRNA expression is common in breast cancer. MMP-1 may be a marker for women at risk for invasive/metastatic breast cancer.

14. SUBJECT TERMS
Breast cancer invasion/metastasis; matrix metalloproteinase-1 mRNA; single nucleotide polymorphism (SNP); Loss of heterozygosity

15. NUMBER OF PAGES
33

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

AD_____

Award Number: DAMD17-00-1-0221

TITLE: Genetic Analysis of a Single Nucleotide Polymorphism in the Matrix Metalloproteinase 1 Promoter in Breast Cancer

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REPORT DATE: July 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
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Table of Contents

Introduction/Aims

Degradation of the extracellular matrix is the *sine qua non* of tumor invasion and metastasis, and it is mediated primarily by matrix metalloproteinases (MMPs). Destruction of the interstitial collagens, types I and III, is a necessary part of this process, since these collagens comprise nearly 30% of body protein and make up the connective tissues through which tumor cells must travel during invasion. Collagen degradation is accomplished primarily by a sub-group of MMPs, the collagenases. Of the three interstitial collagenases that can contribute to invasion, MMP-1 (collagenase-1) is the most ubiquitously expressed and thus, may have the greatest potential for facilitating tumor invasion.

We have found a single nucleotide polymorphism (SNP) in the MMP-1 promoter that enhances transcription of this gene in tumor cells and in normal stromal cells, thereby potentially facilitating cancer progression by more aggressive degradation of the interstitial matrix. The single nucleotide polymorphism (SNP) is located at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a site (5'-AGGA-3'), which binds members of the Ets family of transcription factors, and the absence of the G (5'-AGA-3') lacks this site. The two alleles at this locus, 1G and 2G, are present at approximately equal frequency in normal populations, with 25% being 1G/1G homozygous, 50% being 1G/2G heterozygotes, and 25% being 2G/2G homozygotes. Importantly, the prevalence of the 2G allele was increased significantly in breast tumor cell lines compared to the population gene frequency (P= 0.0001), suggesting a potential role of this SNP in invasive breast cancer. Because MMP-1 expression in breast cancer correlates with an aggressive phenotype, the 2G genotype may serve as a marker for invasive and aggressive disease.

Furthermore, analysis of DNA taken from both normal tissues and from metastatic tumor indicates that some breast cancers display loss of heterozygosity (LOH) at 11q22-23, the chromosomal location of the MMP-1 gene. We have hypothesized that retention of the 2G allele would be associated with aggressive disease, suggesting that these metastatic tumors may have a selective advantage over those with the 1G allele, and that increased expression of MMP-1 may be associated with tumor progression. Thus, we have a structural variation in the MMP-1 gene that may be a useful genetic marker of aggressive disease in breast cancer and that is easily detected.

Accordingly, the specific aims of this study are:

1. To genotype DNA from breast tumors (primary and metastatic, ductal carcinoma *in situ* (DCIS); invasive ductal carcinoma (IDC)) in order to evaluate the frequency of the MMP-1 SNP in the different types and stages of breast cancer.

2. To evaluate, within breast tumor types, Loss of Heterozygosity (LOH) according to stage of disease (primary of metatatic). To this end, normal and tumor tissues from the same patient will be analyzed to determine LOH in the tumor.

3. To examine breast cancer tissues for expression of MMP-1 mRNA. These experiments will determine the level of MMP-1 gene expression, which will be correlated with results of genotyping, tumor grade and stage of disease.

These studies may lead to a new approach for predicting the invasive potential of breast tumors, and may influence the choice of therapies for treating specific breast cancers that contain this variation.

Body

Statement of Work and Progress towards Stated Tasks

* denotes task for year 1

** denotes tasks for year 2

*** denotes tasks for year 3

Aim/Task 1: *To genotype DNA from breast tumors (primary and metastatic, ductal carcinoma* in situ *(DCIS); invasive ductal carcinoma (IDC)) in order to evaluate the frequency of the MMP-1 SNP in the different types and stages of breast cancer.*

* Months 1- 9: Begin initial review of pathology reports on breast cancer specimens housed at the Dartmouth Hitchcock Medical Center. Sort records into type of breast cancer, e.g. primary, metastatic, invasive ductal. Based on review of records, retrieve pathology slide for examination by a pathologist, who will delineate tumor tissue on the slide. Cut slides from block of fixed tissue. COMPLETED

* Months 3 - 9: Begin extraction of tumor tissues from tissue blocks, and perform PCR amplification for the 1G vs. 2G MMP-1 SNP. Whenever possible, amplify normal tissue from the same individual. COMPLETED

** Months 9 - 18: Continue with review of tissue samples. Continue amplification for the MMP-1 SNP in tumor and normal tissue. Begin to analyze data to correlate the 1G vs. 2G SNP with tumor type, primary or metastatic disease, etc. COMPLETED

** Months 18 - 24: Examine data to determine where additional samples are needed in order to substantiate previous findings. COMPLETED

***Months 26 - 36: Finish examination of patient records, PCR amplification of normal and tumor tissues and correlation of SNP with clinical status. COMPLETED

Aim/Task 2: *To evaluate, within breast tumor types, the presence of LOH according to stage of disease. To this end, normal and tumor tissues from the same patient will be analyzed.*

* Months 1- 12: Genotype normal tissue as well as breast tissue from the same individual. Begin to compare the genotype of breast tumors with that of normal tissue taken from the same individual. COMPLETED

* *Months 12 -18: Begin to analyze genotype of tumor tissue vs. normal tissue for LOH. COMPLETED

***Months 18 -24 : Continue analysis of tumor and normal tissue for LOH in tumors. Where LOH has occurred, begin mapping studies to delineate LOH in primary and metastatic tumors. COMPLETED

***Months 24 -36: Finish assaying samples for LOH. Correlate findings with type of breast cancer and state of disease. COMPLETED

Aim/Task 3: *To examine breast cancer tissues for expression of MMP-1 mRNA. These studies will determine the level of MMP-1 gene expression, which will be correlated with results of genotyping, tumor grade and stage of disease.*

** Months 6 - 24: Examine tissue samples from patients for expression of MMP-1 mRNA. COMPLETED

** Months 18 - 24: Begin analysis of data to determine if additional samples are needed from the same or other individuals in order to validate findings. COMPLETED

***Months 24 – 36: Finish expression studies. Correlate these expression data with analysis of the 1G vs 2G SNP and with LOH. COMPLETED

Progress/Accomplishments:

PART 1. "Genetic analysis of a Single Nucleotide Polymorphism (SNP) in the Matrix Metalloproteinase (MMP-1) promoter in breast cancer" (in preparation for *Clinical Cancer Research*)

INTRODUCTION

Degradation of the extracellular matrix is the *sine qua non* of tumor invasion and metastasis, and is mediated primarily by Matrix Metalloproteinases (MMPs), a family of at least 23 enzymes that, collectively, degrade the various components of the matrix. Destruction of the type IV collagen in basement membrane is an essential step in metastasis and this is accomplished primarily by MMP-2 and MMP-9. However, degradation of the interstitial collagens, types I and III, is also a necessary part of the metastatic process, since these collagens comprise nearly 30% of body protein and the connective tissues through which tumor cells must travel during invasion. Of the three interstitial collagenases that can contribute to invasion, MMP-1 (collagenase-1) is the most ubiquitously expressed and thus, has the greatest potential for facilitating tumor invasion. Levels of MMP-1 gene expression are paralleled by MMP-1 protein, which is synthesized and secreted within about 40 minutes. The collagenase protein is secreted in a latent form, and in an *in vivo* environment, serine proteinases and other MMPs activate latent MMP-1.

We have described a single nucleotide polymorphism (SNP) in the MMP-1 promoter that enhances transcription of this gene in both tumor cells and in normal stromal cells, thereby potentially facilitating cancer progression by more aggressive degradation of the interstitial matrix (1). The SNP is located at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a binding site (5'-AGGA-3') for members of the Ets family of transcription factors, and the absence of the G (5'-AGA-3') lacks the binding site. The two alleles at this locus, 1G and 2G, are present at approximately equal frequency in normal populations, with 25% being 1G/1G homozygotes, 50% 1G/2G heterozygotes, and 25% 2G/2G homozygotes (1,2). The 2G allele has been associated with increased incidence of ovarian (3), endometrial (4), and lung (5) cancers, and with the progression of colon (6) cancer and in melanoma (2,7).

The MMP-1 gene is located on chromosome 11q 22-23, a region marked by Loss of Heterozygosity (LOH) in several cancers, including breast cancer (8,9). In a previous study, we demonstrated LOH at this locus in metastatic melanoma (2). We hypothesized that loss of either the 1G or 2G allele would be a random event, but that retention of the 2G allele would enhance the metastatic potential of the tumor. We found that, indeed, retention of the 2G allele was significantly (< 0.04) associated with metastatic melanoma, supporting our hypothesis (2). Breast cancer is also a highly invasive malignancy, and in this study, we investigated the potential role of the 2G allele in this disease. In the present study, we examined the 2G allele in relation to (a) breast cancer

incidence, (b) long-term breast cancer survival, and (c) breast cancer metastasis, as measured by LOH and retention of the 2G allele. We used Laser Capture Microdissection to selectively isolate normal and tumor tissues (Figure 1). In contrast to studies with other aggressive malignancies (2-7), we found no association of the 2G allele with the incidence, survival time of patients, or metastasis of breast cancer. However, LOH at the MMP-1 locus, regardless of which allele is lost or retained, is associated with aggressive and invasive breast cancer, perhaps due to the loss of a tumor suppressor gene that is also present at this locus (8,9). Thus, our data suggest that MMP-1 LOH may be a marker of aggressive and invasive breast cancer.

RESULTS

Possible association of the 2G allele and the incidence of breast cancer. In keeping with several reports linking the 2G allele with the incidence of several cancers (3-5), we examined the association of the 2G allele with the incidence of breast cancer. For this analysis, we compared the 98 breast cancer cases and 75 controls (10) on the basis of MMP-1 promoter polymorphism genotype. The distribution of MMP-1 genotypes for women with breast cancer and for controls is illustrated in Table 1. Our analysis provides no evidence that the 2G allele is over-represented in women with breast cancer, and thus, this allele is not associated with the incidence of these cancers.

Possible association of the 1G allele with long term survival of women with breast cancer. To determine whether the 1G allele, which may have a lower level of MMP-1 gene expression (1), was associated with longterm breast cancer survival, we compared genotypes of the 77 long term survivors to those of 72 controls enrolled in the case-control study (10). The distribution of MMP-1 genotypes for these two groups is shown in Table 2. While there is an apparent slight over-representation of the 1G allele in the long-term survivors, this finding is not statistically significant ($p=0.362$) and therefore, neither allele is associated with breast cancer survival.

Possible association of the 2G allele with breast cancer metastasis. Although loss of either the 1G or 2G allele is a random event, retention of the 2G allele (as opposed to retention of the 1G allele) might confer a relative advantage to the tumor with respect to invasion and metastasis (2). This would, therefore, result in an apparent higher prevalence of metastases that have retained the 2G allele than those retaining the 1G allele. We tested this hypothesis by measuring LOH at the MMP-1 locus in women with breast cancer who are 1G/2G heterozygotes.

Figure 2 shows an autoradiogram representing the standard curve from each amplicon from which the degree of LOH in patient samples was determined, as well as two representative metastatic breast tumors that have undergone loss of the 1G allele. In this group of 96 patients, there were 47 heterozygotes (Table 3), but two of these heterozygotes had tumors that would not amplify. Thus, 45 tumors were examined for LOH, which was present in 19 tumors (42%) (Figure 3). Of these 19, 10 retained the 2G allele and 9 retained the 1G allele, demonstrating that the 2G allele is not selectively associated with breast cancer metastasis.

Analysis of LOH and characteristics of breast tumors in selected patients. We wanted to determine if LOH arose in the primary tumor, or was a later event that occurred in the metastatic tumor. We examined normal tissue, primary tumor and metastatic tumor tissues from seven of the 19 heterozygotic patients who displayed varying degrees of LOH for either the 1G or 2G allele. In all of these seven pients, LOH occurred in the primary tumor and, for any given patient, the degree of LOH was remarkably constant throughout all the tumor samples tested (Figure 4).

Finally, we correlated LOH with the characteristics of the breast tumor in the 19 individuals with LOH of either the 1G or 2G allele (Figure 3 and Table 4). While there is no association with ER/PR status or with molecular markers such as C-erbB-2, her2/neu and p53, 16 of these patients had tumors of intermediate/high grade (scoring 6 or greater), as measured by the Scarff-Bloom-Richardson scale, suggesting a possible link between tumor grade and LOH at the 11q22-23 locus and the MMP-1 gene. LOH is usually thought to be associated with the loss of a tumor suppressor

gene at the 11q 22-23 locus (8,9), and the aggressive nature of breast cancer in these patients may be due to the loss of this gene, rather than to the loss of a MMP-1 allele. However, these tumors may express high levels of MMP-1, regardless of genotype, as suggested by our studies (Brinckerhoff et al., see PART 2, below). Thus, LOH of the MMP-1 gene may be a marker that identifies aggressive breast cancers.

PART 2. Matrix Metalloproteinase-1 (MMP-1) gene expression in human breast cancer progression (in preparation for *Oncogene*)

INTRODUCTION

As described above, our analysis of samples from population-based breast cancer cases and controls showed no evidence that the 2G allele is associated with breast cancer incidence. In addition, the comparison of long term survivors with non-cancer controls provided little evidence that the 1G allele is selectively associated with long term survival. Finally, our data did not show that the 2G allele is selectively linked to breast cancer metastasis.

Thus our findings indicate that the 1G/2G SNP in the MMP-1 promoter does not contribute significantly to breast cancer incidence or outcome, and that there is no selective pressure on either the 1G or 2G allele in this disease. This is in contrast to five other cancers in which the 2G allele and heightened MMP-1 expression contribute to either the incidence or progression of cancer (2-7). However, our results do not rule out a substantial role for MMP-1 in the development of breast cancer and its spread since modulation of MMP-1 expression may occur independently of the 1G/2G promoter genotype (11).

Indeed, the 2G allele is not the only mechanism for achieving high levels of MMP-1 gene expression (11,12) If the 2G allele is not present, cells may use alternative cis-acting sequences in the promoter to increase gene expression, as has been described for a melanoma cell line that is homozygous for the 1G allele and that expresses high levels of MMP-1 constitutively (12). Thus MMP-1 expression in tumor tissues may be high, regardless of the SNP genotype. To test this hypothesis, we used quantitative real-time RT-PCR to examine breast tissue from normal tissue and from various stages of cancer progression (atypical ductal hyperplasia (ADH); ductal carcinoma *in situ* (DCIS); and invasive ductal hyperplasia (IDC)) for levels of MMP-1 mRNA. We found that (a) MMP-1 gene expression is common in breast cancer, and increases as the tumor stage progresses from ADH to DCIS to IDC, (b) MMP-1 levels increase as tumor grade increases, and (c) high levels of MMP-1 are not restricted to tissues from individuals with the 2G genotype,. Thus, MMP-1 appears to contribute to the invasive pathology of breast cancers.

RESULTS

Patient population and tumor characteristics. Tumors from 32 breast cancer patients were characterized from stage, tumor grade, ER/PR status and Her2 expression (Table 5). Normal tissue was also isolated, and tissues at various stages of tumor progression were captured simultaneously by laser dissection from the same patient for pathologic characterization, genotyping and MMP-1 expression. Patients ranged in age from 28 to 79 years, with a mean of 44 years. Of these, 21 were ER positive, six were ER negative and no data were available on four individuals. Eighteen were PR positive, and eight were PR negative. Eight were HER2 positive and 22 had positive lymph nodes, while eight were node-negative, with no lymph node dissection performed on one patient. Thus, we have a wide sampling to breast tissues from which to analyze MMP-1 expression.

MMP-1 SNP genotypes of patient population. DNA was amplified for genotyping the MMP-1 SNP from 23 of the 32 patients presented in Table 5, along with 12 additional patients (Table 6). Sufficient DNA was not available for four samples. In agreement with previous studies (1,2), approximately 50% of the individuals were heterozygotic, and approximately 25% were homozygotic for the 1G allele. Although 2G homozygotes accounted for only 11% of the patients

and would appear to be under-represented, this is not statistically significant.

MMP-1 expression in breast cancer patients. We obtained MMP-1 expression data on samples from 41 patients, including the 32 shown in Table 5 (and see Appendices). Relative levels of MMP-1 expression were determined by comparing the threshold cycle (Ct) for normal tissue to that of the tumor tissue (13). Since the threshold cycle is defined as the point where fluorescence of an unknown sample moves above background, a Ct that is never above background indicates that there is no target mRNA present. If by cycle 40 no MMP-1 mRNA was recognized then it was accepted that there is either no expression of MMP1, or only a few copies (below the threshold of amplification in a PCR). Thus if one patient has MMP-1 showing up at Ct 40 in their normal tissue, and Ct 25 in their tumor, that means that there is 2^15 times as much MMP-1 message in the tumor than in the normal. Because 40 cycles is the threshold for all samples, we made relative comparisons among the samples for the level of MMP-1 expression.

MMP-1 expression in normal tissue and tumor stage: ADH, DCIS and IDC. Five of the 41 patients showed no MMP-1 expression regardless of tumor stage or grade (data not shown). Interestingly, substantial levels of MMP-1 (Ct less than 37) were detected in 11 of the 41 samples, approximately 25% (data not shown), suggesting that some level of MMP-1 expression may be a part of normal breast tissue physiology, particularly in premenopausal women (See Table 5 and Appendices). When DCIS tumor tissue was taken from some these patients, no MMP-1 expression was detected. This may be a function of the fact that the sample of DCIS were taken from the middle of the lesion, on order to avoid any contamination with normal tissue. However, these relatively deep cells may not be stimulated with the growth factors and cytokines released by stromal cells and macrophages known to stimulate MMP-1 expression (11).

As the tumors progressed from ADH to DCIS to IDC , MMP-1 expression increased relative to normal tissues in ADH and then continued to remain high or increase further in DCIS and IDC (Figure 5). Of the seven patients with tissue samples from all three stages of breast cancer progression, two exhibited a decrease in MMP-1 expression in tumor tissues, compared to normal tissue, possibly due to sampling (see above). However, the remaining five showed increases of 10 to 100 times greater than that seen in normal tissue in ADH, DICS and ID and in the later lesions. This finding suggests that MMP-1 expression increases early in breast cancer and remains high.

MMP-1 expression and tumor grade. We analyzed whether MMP-1 expression increased as tumor grade in DCIS and IDC increased from low to high (Figure 6). The figure shows that as the grade of the tumor became more aggressive, the level of MMP-1 expression also increased. The increase was not particularly noteworthy as the stage progressed from DCIS to IDC. However, as the grade changed from low to intermediate to high, the level of MMP-1 expression increased concomitantly. A low grade tumor showed a 10 to 100 fold increase over normal, while the increases for an intermediate grade ranged from 100 to 1000 –fold. Of the seven patients with high grade tumors, five showed increases greater than 1000 fold over normal tissue. One patient showed essentially no increase over normal tissues; however this patient was pregnant when the tumor samples were taken, suggesting that this condition might influence MMP-1 expression.

MMP-1 expression and genotype. Lastly, we measured the correlation between MMP-1 expression in IDC and the 1G/2G genotype (Figure 7). Of the seven 1G/1G homozygotes analyzed, 6 showed levels of MMP-1 expression at least 1000-fold greater than the normal tissue taken from these same individuals. Further, of the 14 individuals who were either 1G/2G heterozygotes or 2G/2G homozygotes, seven showed these same high levels of increase. Of the seven who did not show a large fold increase over normal, four displayed high levels of MMP-1 in their normal tissue, suggesting that the MMP-1 gene had already been activated and that perhaps the presence of the 2G allele may influence basal expression. Thus, the 2G genotype does not correlate with levels of MMP-1 expression in breast cancer progression, a finding that is supported by the studies outlined in PART 1 of this report, above.

METHODS

Clinical samples. Specimens of breast tissue from 41 women were obtained from the Massachusetts General Hospital between 1998 and 2000 (13). Tissue specimens demonstrating one or more pathologic lesions (ADH, DCIS, IDC) were used, along with normal control tissue from the same patient. Pathologic staging, histologic grade, estrogen receptor and progesterone receptor status and Her-2 expression were determined as previously described (13). Briefly, DCIS was diagnosed as described by the European Classification Scheme, with low grade characterized by clinging, cribriform or micropapillary proliferation of small cells with rare mitosis, while high-grade DCIS has a solid or clinging proliferation of large cells with frequent mitoses. IDC was classified by the Nittingham combined histologic grade. ER and PR status were determined by immunohistochemistry and Her-2 expression was measured by immunohistochemistry or in situ hybridization. The study was approved by the Massachusetts General Hospital human research committee and follows the National Institutes of Health human research study guidelines.

Laser Capture Microscopy, and RNA Isolation and Amplification. Using consecutive tissue sections, normal, ADH, DCIS and IDC samples were laser capture dissected in triplicate as described (13). Normal tissue that was dissected was a minimum of 0.3 cm from any malignant tissues. Malignant epithelial cells were dissected from areas of tumor where the grade of tumor was uniform. RNA was extracted and T7-based RNA amplification was carried out with the RiboAmp kit (13), as described by the manufacturer. To assure sufficient amounts of RNA for amplication, a second round of amplification was carried out on all samples.

Quantitative Real-time PCR. We captured approximately 40,000 normal breast epithelial cells or abnormal epithelial cells from DCIS or IDC cases. Total RNA was isolated and cDNA was synthesized and converted to dsDNA as described (13). Briefly, to validate the studies with amplified RNA, 2 ug of amplified RNA from each sampled of micro-dissected tissue was converted into double-stranded cDNA. cDNA from non-amplified samples and from amplified samples was quantitated with PicoGreen (Molecular Probes) with a spectrofluorometer (Molecular Probes). Each reaction was carried out in triplicate with 2.5 ng of cDNA from each sample as template. The relative standartd curve methods was used for linear regression of unknown samples and the data are presented as fold change between samples.

The threshold cycle for fluorescence of an unknown sample to be classified as above background is Ct of 40. If no MMP-1 mRNA was detected by cycle 40, no MMP-1 mRNA, no target mRNA present and there is no expression of MMP1, or only a few copies (below the threshold of amplification in a PCR). Thus if one patient has MMP-1 appearing at Ct 40 in the normal, and Ct 25 in the tumor, there is 2^15 times as much MMP-1 message in the tumor than in the normal.

DATA FOR PART 1.

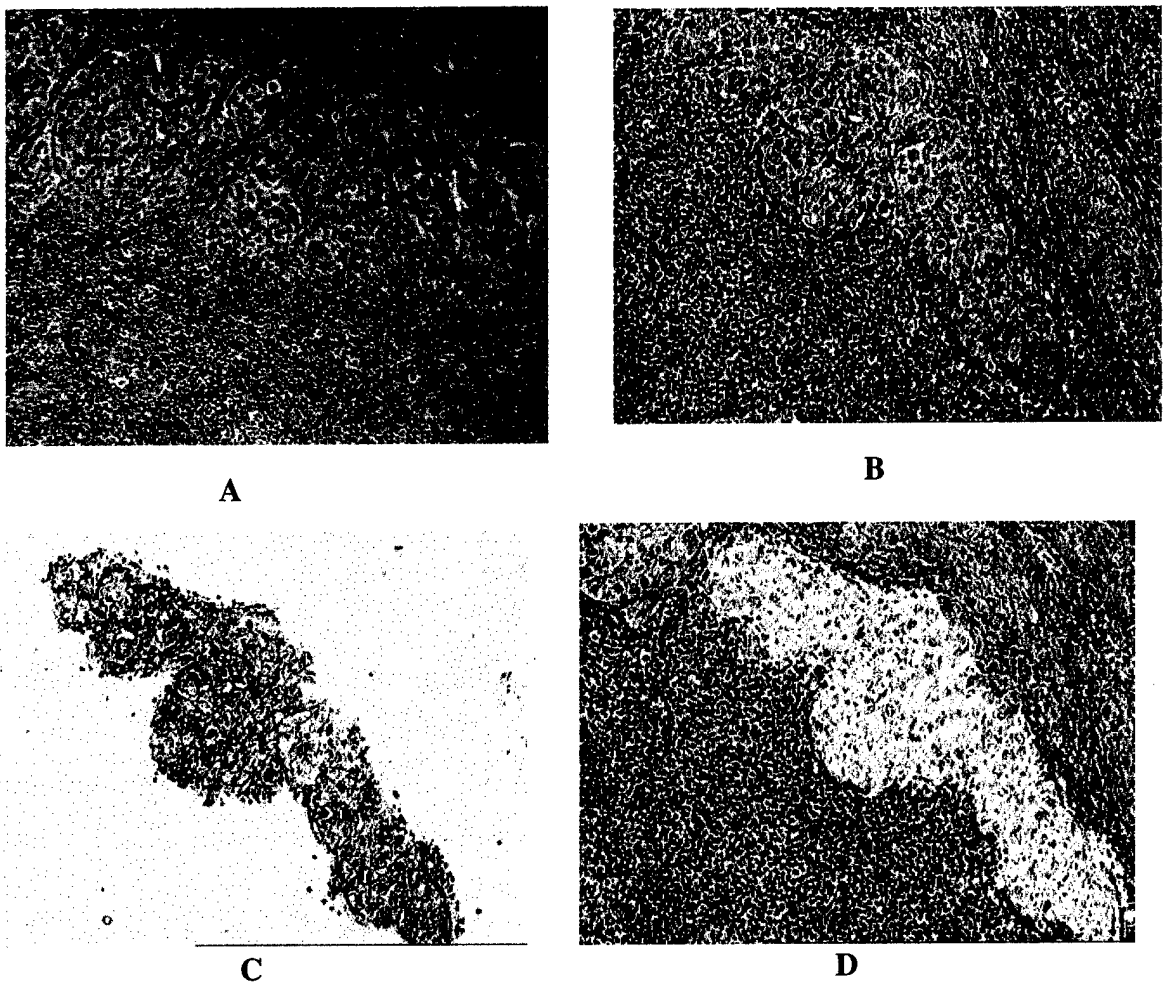


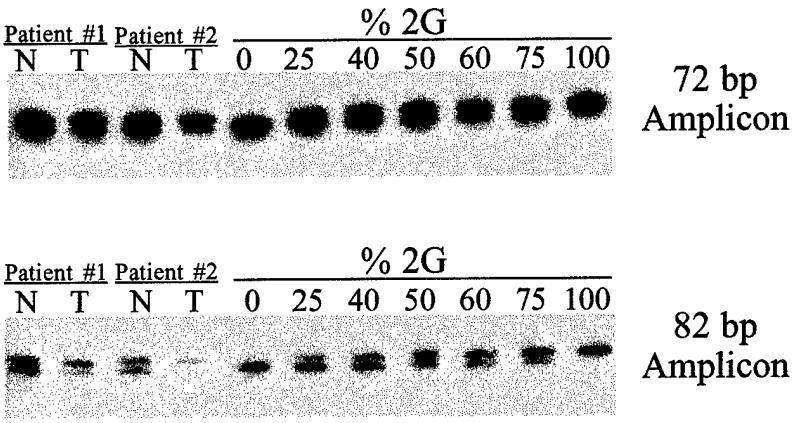
Figure 1: Laser Capture Microdissection using the Arcturus Pix-Cell II. **Figure 1A shows breast carcinoma metastatic to lymph node, H and E stain. Fgure 1B shows a serial section of the same tissue stained with Methyl Green. Figure 1C shows the area of tumor which has been microdissected away from the normal tissue, which is shown in figure 1D, after dissection.**

Genotype	Normal (N=72)	Breast Cancer (N=98)
1G/1G	27.8 % (N=20)	29.6 % (N=29)
1G/2G	45.8 % (N=33)	46.9 % (N=46)
2G/2G	26.4 % (N=19)	23.5 % (N=23)

Table 1: MMP-1 Genotype and the incidence of cancer. **Data are from women enrolled in a statewide (New Hampshire) population-based case-control study of breast cancer.**

Genotype	Normal (N=72)	Survivors (N=77)
1G/1G	27.8 % (N=20)	29.9 % (N=23)
1G/2G	45.8 % (N=33)	53.2 % (N=41)
2G/2G	26.4 % (N=19)	16.9 % (N=13)

Table 2: MMP-1 genotype and long-term survival of patients with breast cancer. **Long term survivors were identified as being at least 5 years post-diagnosis, not currently receiving cancer treatment and disease-free. While there is an apparent over-representation of the 1G allele in the long-term survivors, this finding does not achieve statistical significance (p=0.362).**



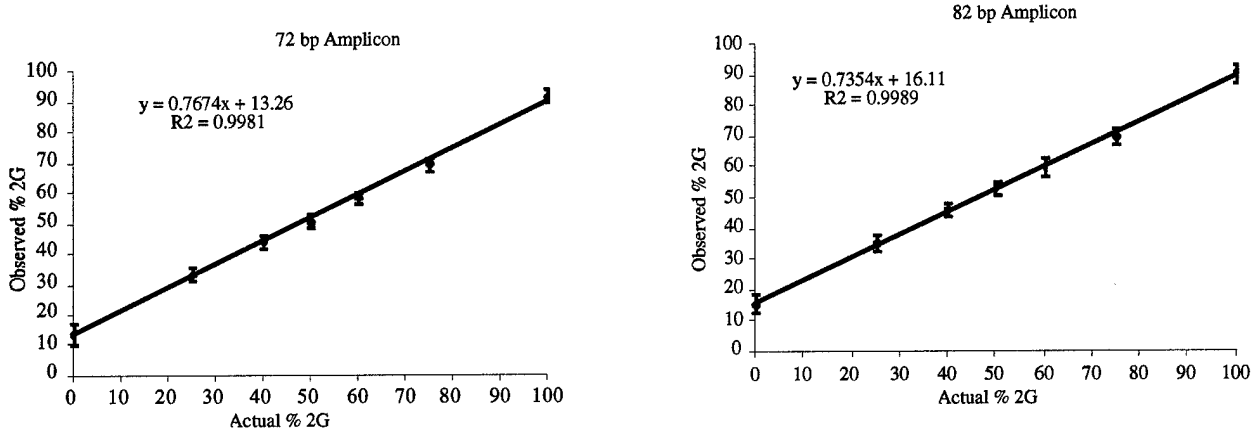


Figure 2. Radiographic Assay for LOH. **A: Representative gels showing the standard curve and 2 patients whose tumors have undergone loss of the 1G allele. (N=normal tissue; T= metastatic tumor tissue). B: Data from 17 experiments were averaged and plotted above. The error bars represent +1SD. This figure illustrates the high level of reproducibility of the LOH assay.**

Genotype	N=96
1G/1G	25.0 % (N=24)
1G/2G	49.0 % (N=47)
2G/2G	26.0 % (N=26)

Table 3. Genotype of breast cancer patients analyzed for LOH. **LOH cases are the cases identified for this study and were genotyped using normal tissue from the biopsy specimens.**

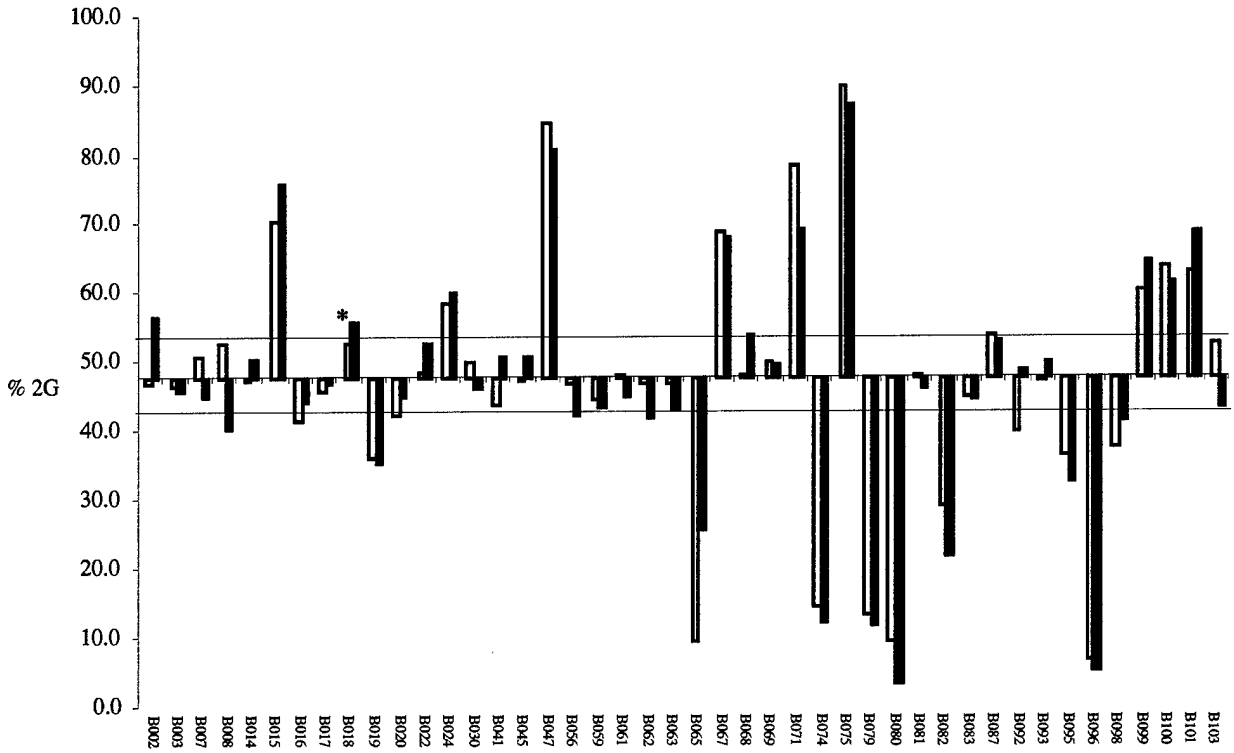


Figure 3: LOH data on all heterozygote patients. **Data show the %2G in the metastatic tumor for the 45 heterozygous patients studied. The white bars represent the 72 bp primer set and the black bars represent the 82 bp primer set. The x-axis crosses at 47.7%, which is the average calculated %2G for heterozygotes over 17 experiments. The lines represent ±2SD of that average. Loss of heterozygosity is defined as being outside those lines. DNA from patient B018 (*) was redissected and amplified and found not to have undergone LOH.**

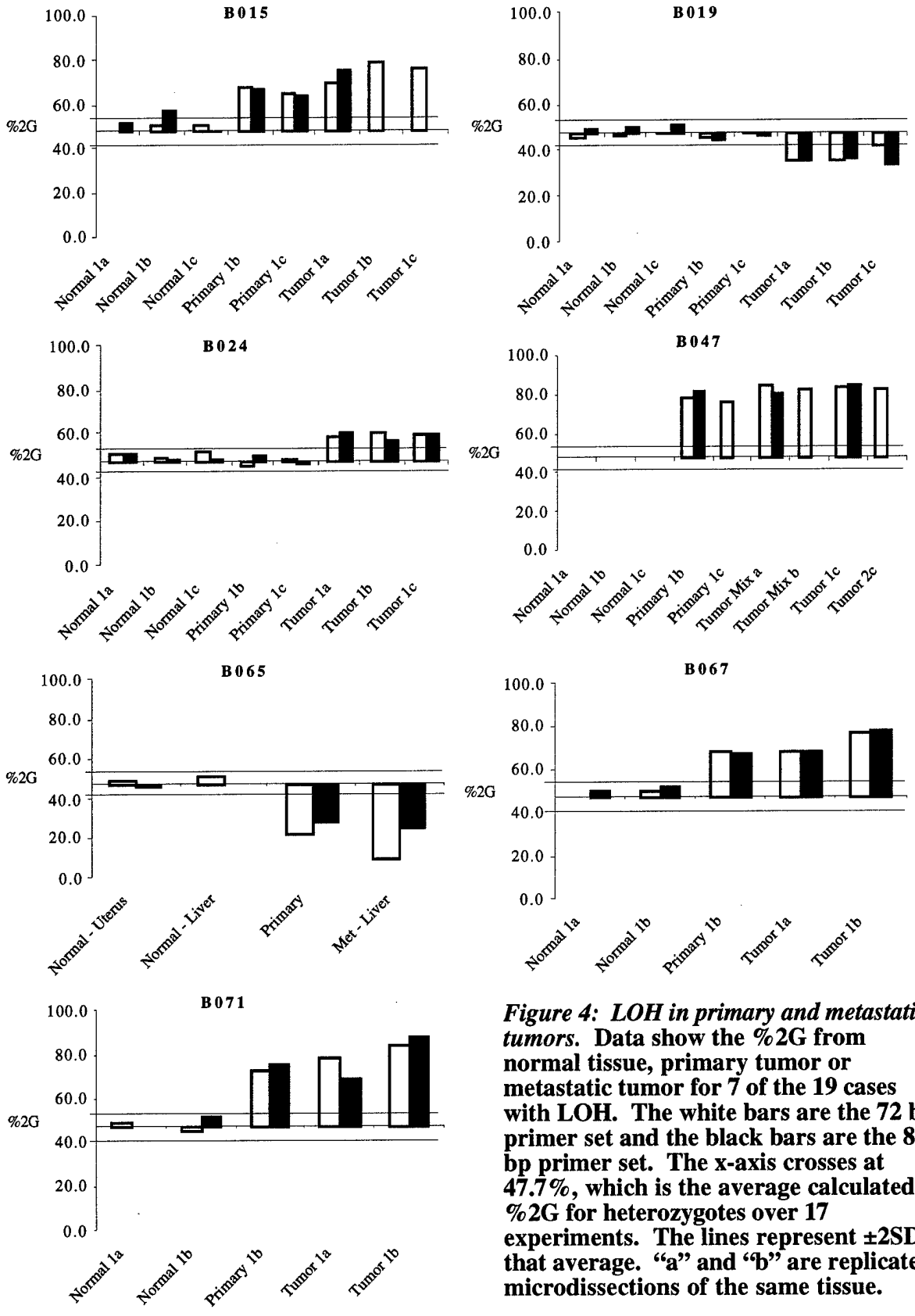


Figure 4: LOH in primary and metastatic tumors. **Data show the %2G from normal tissue, primary tumor or metastatic tumor for 7 of the 19 cases with LOH. The white bars are the 72 bp primer set and the black bars are the 82 bp primer set. The x-axis crosses at 47.7%, which is the average calculated %2G for heterozygotes over 17 experiments. The lines represent ±2SD of that average. "a" and "b" are replicate microdissections of the same tissue.**

Patient	Histologic type	Tumor Grade	SBR Score	Total # of nodes sampled	# positive for tumor	ER	PR	C-erbB-2 Protein
B015	Infiltrating Ductal	Intermediate	7	13	7	+	+	Negative
B019	Infiltrating Ductal	High	8	17	7	+	+	3+ membranous
B024	Infiltrating Ductal	High	9			-	-	Negative
B047	Infiltrating Ductal	High	9			-	-	Negative
B065	Poorly Differentiated		III of III	16	13	+	+	weakly positive
B067	Infiltrating Ductal	Intermediate	7	14	5	+	-	Negative
B071	Infiltrating Ductal	Low	5	11	11	+/-	+	Negative
B074	Infiltrating Ductal	Intermediate	6	20	2	+	-	Background Cytoplasmic
B075	Infiltrating Ductal	Intermediate	6	9	4	-	-	Focal
B079	Infiltrating Ductal	Intermediate	7	11	11	-	-	
B080	Infiltrating Ductal	Intermediate	7	26	3	-	-	
B082	Infiltrating Ductal	Poorly Differentiated	9	16	1	+	+	
B087	Infiltrating Ductal	Intermediate		13	6	+	+	2+ Positive
B095	Infiltrating Ductal	High	8	2	2	+	+	1+ mebranous
B096	Infiltrating Ductal	Intermediate	7	1	0	+	-	2-3+ Membranous
B098	Infiltrating Ductal	Low	4	4	1	+	+	1+ Membranous
B099	Infiltrating Ductal	Intermediate	7	3	1	+	+	
B100	Infiltrating Ductal	Intermediate	6	9	4	+	+/-	No Membranous
B101	Infiltrating Ductal	Intermediate	6	18	18	+	+	No Membranous

Table 4: Data on tumor characteristics for patients with LOH of MMP-1.

DATA FOR PART 2.

Case ID	stages captured	age	ER	PR	Her2	Node	genotype
14	DCIS (III), IDC (III)	48	pos	pos	pos	pos	N/A
30	DCIS (III), IDC (III)	47	neg	neg	neg	pos	2G/2G
41	DCIS (III), IDC (III)	55	pos	pos	ND	neg	1G/2G
43	DCIS (II), IDC (II)	53	pos	neg	neg	pos	1G/2G
44	DCIS (III), IDC (III)	28	pos	pos	neg	neg	1G/1G
45	DCIS (I)	36	pos	neg	neg	neg	1G/2G
57	ADH, DCIS (I)	34	ND	ND	ND	neg	N/A
65	DCIS (III), IDC (III)	39	pos	pos	neg	neg	N/A
79	ADH, DCIS (I) IDC (I)	54	pos	pos	neg	pos	1G/1G
88	DCIS (III), IDC (III)	35	pos	pos	ND	pos	1G/2G
96	DCIS (III), IDC (III)	31	neg	neg	neg	pos	1G/2G
97	DCIS (III), IDC (III)	79	neg	neg	pos	pos	N/A
102	DCIS (I), IDC (I)	55	pos	neg	neg	pos	1G/1G
112	DCIS (III), IDC (III)	31	neg	pos	neg	pos	1G/1G
121	DCIS (II), IDC (II)	45	pos	pos	pos	pos	2G/2G
130	DCIS (II), IDC (II)	54	pos	pos	neg	pos	1G/2G
131	ADH, DCIS (II), IDC (II)	37	pos	pos	pos	pos	2G/2G
133	DCIS (III), IDC (III)	44	neg	neg	pos	pos	2G/2G
148	DCIS (II), IDC (II)	42	pos	pos	neg	pos	1G/2G
152	DCIS (III)	55	ND	ND	ND	neg	1G/2G
153	DCIS (II)	46	pos	pos	pos	pos	N/A
169	DCIS (II), IDC (II)	34	pos	pos	neg	pos	1G/1G
170	DCIS (II), IDC (II)	44	pos	pos	ND	pos	1G/2G
173	DCIS (I), IDC (I)	52	pos	pos	neg	neg	1G/2G
178	DCIS (III), IDC (III)	43	pos	pos	pos	pos	1G/1G
179	DCIS (III), IDC (III)	37	neg	neg	pos	pos	N/A
180	ADH, DCIS (I), IDC (I)	46	pos	pos	ND	pos	1G/2G
183	DCIS (II)	46	ND	ND	ND	pos	N/A
191	ADH, DCIS (II)	43	ND	ND	ND	ND	1G/1G
193	ADH, DCIS (I), IDC (I)	45	pos	pos	neg	pos	1G/1G
198	DCIS (II), IDC (II)	30	pos	pos	ND	neg	N/A
210	ADH, DCIS (I)	62	ND	ND	ND	neg	N/A

ND = not detected
N/A = not available
DCIS = ductal carcinoma in situ
ADH = atypical ductal hyperplasia
IDC = invasive ductal carcinoma
ER = estrogen receptor
PR = progesterone receptor

Table 5. Age and tumor characteristics of 32 patients with breast cancer.

Not Obtainable	1G/2G	2G/2G	1G/1G
Patient 5	Patient 11	Patient 39	Patient 44
65	41	60	79
183	43	121	102
184	45	131	112
total = 4	53	total = 4	133
11.4%	72	11.4%	169
	75		178
	88		191
	89		193
	96		total = 9
	122		25.7%
	130		
	148		
	152		
	153		
	170		
	173		
	total = 18		
	51.4%		

Table 6. MMP-1 genotyping of 35 breast cancer patients

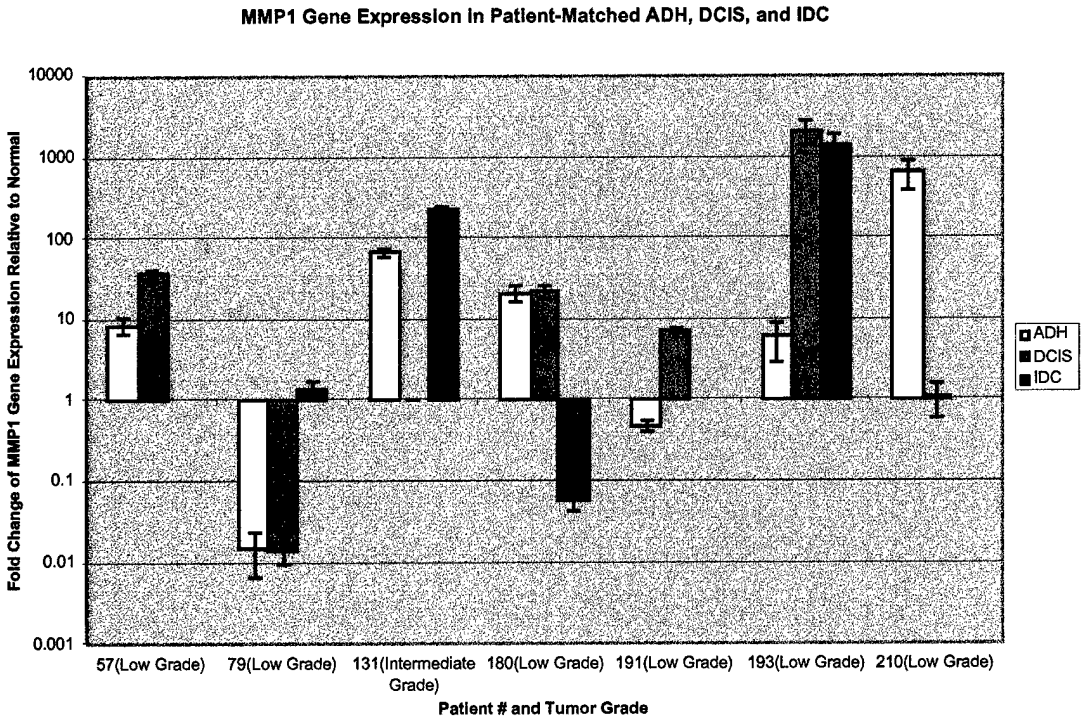


Figure 5. MMP-1 expression in patients with progressing breast cancer: ADH, DCIS and IDC.

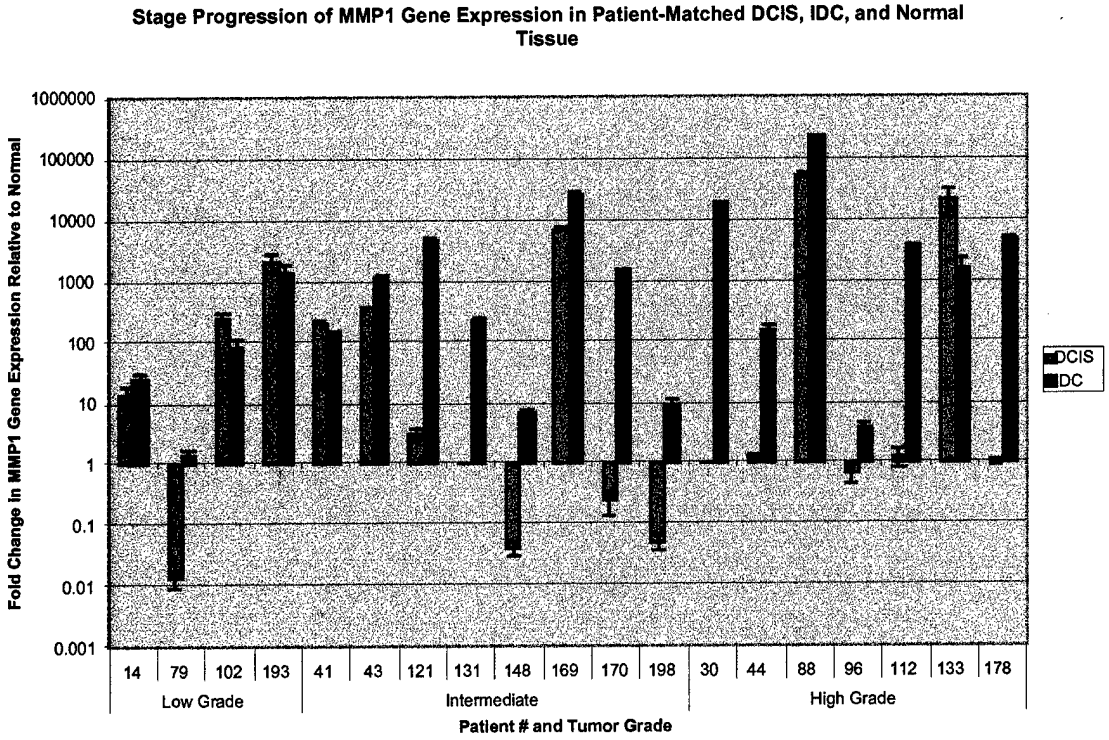


Figure 6. MMP-1 expression in low, intermediate and high grade breast tumors.

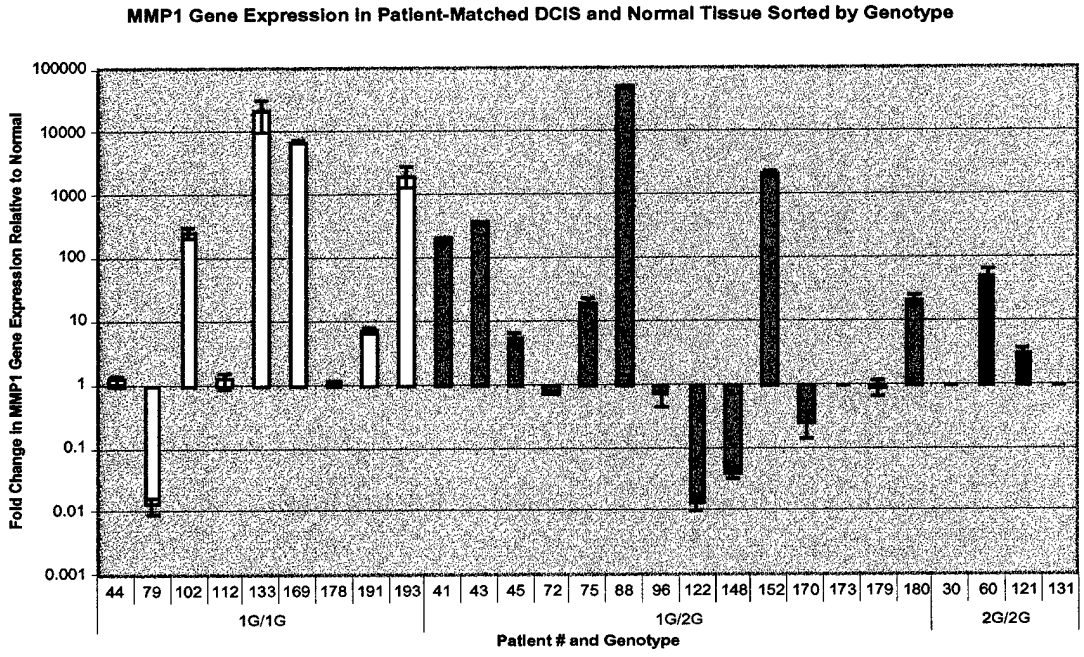


Figure 7. MMP-1 expression in breast cancer by MMP-1 SNP genotype.

Key Research Accomplishments

- Genotyped DNA from 82 breast cancer patients and 75 controls to compare the prevalence of the 2G allele in the incidence of breast cancer.
- Genotyped DNA from 75 long term breast cancer survivors and 75 controls for the prevalence of the 1G allele and breast cancer survival.
- Genotyped normal and tumor DNA from 96 patients with metastatic breast cancer
- Examined 45 of these for LOH at the MMP-1 locus.
- Detected LOH in 19 (42%); retention of the 2G allele in 10 samples, retention of the 1G allele in 9 samples.
- Characterized tumors from 32 patients; genotyped normal and tumor (ADH, DCIS, IDC) DNA from 35 breast cancer patients; examined tissues from 41 patients for MMP-1 expression by real-time quantitative PCR. Found MMP-1 expression to be a common event and to increase as tumor grade increased.

Reportable Outcomes

Personnel supported by this grant:

Constance E. Brinckerhoff, PhD. PI
Dorothy Belloni, B.S, Research Assistant

Abstract: submitted to DoD, April, 2002; presented in September, 2002 at DoD meeting.

GENETIC ANALYSIS OF A SINGLE NUCLEOTIDE POLYMORPHISM IN THE MATRIX METALLOPROTEINASE 1 (MMP-1) PROMOTER IN BREAST CANCER

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Death from breast cancer results from tumor metastasis, and the *sine qua non* of metastasis is degradation of the extracellular matrix, a process that is mediated primarily by Matrix Metalloproteinases (MMPs). Destruction of the interstitial collagens, types I and III, is a necessary part of the process, since these collagens comprise nearly 30% of body protein and the connective tissues through which tumor cells must travel during invasion. Of the three interstitial collagenases that can contribute to invasion, MMP-1 (collagenase-1) is the most ubiquitously expressed and thus, has the greatest potential for facilitating tumor invasion.

We have found a single nucleotide polymorphism (SNP) in the MMP-1 promoter that enhances transcription of this gene in tumor cells and in normal stromal cells, thereby potentially facilitating cancer progression by more aggressive degradation of the interstitial matrix. The SNP is located at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a binding site (5'-AGGA-3') for members of the Ets family of transcription factors, and the absence of the G (5'-AGA-3') lacks the binding site. The frequency of this SNP in the population is 25% =1 G, 25% =2 G, and 50% = heterozygous. The 2G allele has been associated with increased incidence or progression in five cancers: ovarian, endometrial, melanoma, colon and lung, and this study investigates its potential role in breast cancer.

We first examined the association of the 2G allele with the incidence of breast cancer. The 1G/2G genotype of 157 women was evaluated by PCR of DNA obtained by buccal swabs (Cancer

Epidemiology, Biomarkers, and Prevention. 10: 687, 2001). Of these, 82 were invasive breast cancer cases representing ductal, lobular, and ductal with a lobular component, and 75 were from normal controls. The genotypes for women with cancer were: 29% = 1G homozygous, 21% = 2G homozygous and 50% = 1G/2G heterozygous, and the types for the control women were: 27% =1G homozygous, 27% = 2G homozygous and 47% = 1G/2G heterozygous. Thus, there appears to be no link between the 2G genotype and the incidence of breast cancer.

We next investigated the association between the 2G allele and breast cancer metastasis and progression. We have begun our analysis with invasive ductal carcinoma, since this is the most common type of breast cancer. Based on our previous experiences with metastatic melanoma (Am. J. Pathol. 158: 691, 2001), we genotyped DNA from 58 patients with overt metastatic breast cancer and found no deviation from control values, suggesting that the 2G allele does not favor metastasis. We have also analyzed tumor tissue from the heterozygotes for Loss of Heterozygosity (LOH) at the 11q 22-23 locus, a common site for LOH in breast cancer and the location of the MMP-1 gene, since we hypothesized that retention of the 2G allele after LOH provided tumors with an advantage for progression. We used our ^{32}P PCR assay with overlapping sets of primers (82 bp or 72 bp) to amplify DNA from the tumor tissue. Of 24 heterozygotes, we observed LOH in only 5, with retention of the 2G allele in 3 cases. Thus, additional samples need to be analyzed before we can conclude that the presence of the 2G allele in the MMP-1 promoter signifies greater invasive potential of the tumor. This allelic variation may be a meaningful genetic marker that can help identify those women at higher risk for invasive/metastatic disease, and may have important implications for the diagnosis and treatment of certain types of breast cancer.

Manuscripts:

1. Dorothy R. Belloni, Sharon M. Tobias, Linda T. Titus-Ernstoff, Walter W. Noll, and Constance E. Brinckerhoff: "Genetic analysis of a Single Nucleotide Polymorphism (SNP) in the Matrix Metalloproteinase-1 (MMP-1) promoter in breast cancer" in preparation for *Clinical Cancer Res.* (See DATA PART 1)

2. Justin J. Gaudet, Colby A.Wyatt, Dennis C. Sgroi and Constance E. Brinckerhoff: "Matrix Metalloproteinase-1 (MMP-1) gene expression in human breast cancer progression" in preparation for *Oncogene.* (See DATA PART 2)

Conclusions

1. The study progressed in a timely manner and met its goals. Two peer-reviewed publications will result from this study.

2. The data obtained support the hypothesis that the 2G allele does not predispose women to breast cancer, is not correlated with shortened survival time and is not associated with and enhanced invasion of established disease. Thus, there appears to be no selective pressure that favors the 2G allele in breast cancer.

3. LOH at the MMP-1 locus (11q 22-23) is associated with aggressive and invasive disease, regardless of the MMP-1 allele that is retained. While high MMP-1 levels may be expressed in breast cancer, the loss of a tumor suppressor gene at this same locus may facilitate rapid tumor growth. Thus, LOH of MMP-1 may be a marker for tumors at risk for aggressive and invasive behavior.

4. The data also indicate that MMP-1 expression is a common and early event in breast cancer, and that the levels of expression increase as the disease progresses from ADH to DCIS to IDC. Patients with either allele produce increasing amounts of MMP-1 as the disease progresses.

5. The "so what" of this study are the facts that (a) the 2G allele does not seem to predispose Caucasian women to breast cancer, a finding that contrasts with studies on ovarian (5), endometrial (6) and lung (7) cancers, (b) the 2G allele is not selectively associated with breast cancer progression, a finding that also contrasts with two other cancers: melanoma and colon cancer (c) MMP-1 mRNA is expressed by breast cancer tissues, regardless of MMP-1 genotype (d) this level of expression is high and appears to increase above levels seen in normal tissue at an early stage in breast cancer (ADH), and (d) MMP-1 heterozygotes that have undergone LOH at the MMP-1 locus have aggressive disease. Since this locus harbors a tumor suppressor (gene), loss of both copies of the tumor suppressor may signify proliferating and invasive disease. Our data suggest that since the 2G allele does not mediate increased MMP-1 expression, compensatory mechanisms are present to drive MMP-1 expression in cells containing either allele, as has been described in one melanoma cell line (8). Since LOH at the MMP-1 locus is associated with aggressive disease, the MMP-1 SNP may be a meaningful marker to identify women with aggressive disease. *These findings are important in understanding the pathogenesis of breast cancer and in designing therapies to prevent metastasis.*

References

1. Rutter, J.L., Mitchell, T.I., Buttice, G., Meyers, J., Gusella, J.F., Ozelius, L.J. and Brinckerhoff, C.E. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res. 58: 5321-5325, 1998.

2. Noll, W.W., Belloni, D.R., Rutter, J.L., Storm, C.A., Schned, A. R., Titus-Ernstoff, L., Ernstoff, M.S. and Brinckerhoff, C.E. Loss of heterozygosity on chromosome 11q.22.23 in melanoma is associated with retention of the insertion polymorphism in the matrix metalloproteinase 1 promoter. Am. J. Pathol. 158: 691-697, 2001.

3. Kanamori, Y., Matsushima, M., Minaguchi, T., Kobayashi, K., Sagae, S., Kudo, R., Terakawa, N., and Nakamura, Y. Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res. 59: 4225 – 4227, 1999.

4. Nishioka, Y., Kobayashi, K., Sagae, S., Ishioka, S., Nishikawa, A., Matsushima, M., Kanamori, Y., Minaguchi, T., Nakamura, Y., Tokina, T. and Kudo, R. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter in endometrial carcinomas. Jpn. J. Cancer Res. *91:* 612– 615, 2000.

5. Zhu, Y., Spitz, M.R. Lei, L., Mills G.B. and Wu X. A single nucleotide polymorphism in the matrix metalloproteinase –1 (MMP-1) promoter enhances lung cancer susceptibility. Cancer Res. 61:7825-7829, 2001.

6. Gilhardi, G., Bioni, M.L., Mangoni, J., Leviti, S., DeMonti, M., Guagnellini, E. and Scorza, R. Matrix Metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. Clin Cancer Res. 2001;7:2344-2346

7. Ye, S., Dhilon, s., Turner, S.J., Bateman, A.C., Theaker, J.M., Pickering, R.M., Day, I. And Howell, W.M. Invasiveness of cutaneous malignant melanoma is influenced by Matrix Metalloproteinase 1 gene polymorphism. Cancer Res. 2001;61:1296-1298

8. Driouch, K., Briffod, M., Bieche, I., Champeme, M.-H. and Lidereau, R. (1998) Location of several putative genes possible involved in human breast cancer progression. Cancer Res. 58: 2081-2086.

9. Laake, K., Launonen, B., Niederacher, D., Gudlaugsdottir, S., Seitz, S., Rio, P., Champeme, M.-H., Bieche, I., Birnbaum, D., White, G., Sztan, M., Sever, N., Plummer, S., Osorio, A., Broeks, A., Huusko, P., Spurr, N., Borg, A., Cleton-Jansen, A.-M., van't Veer, L., Benitez, J., Casey, G., Peterlin, B., Olah, E., Varley, J., Bignon, Y.-J., Scherneck, S., Sigurdardottir, V., Lidereau, R., Eyfjord, J., Beckmann, M.W., Winqvist, R., Skovlund,. E. and Borresen-Dale, A.-L., Breast Cancer Somatic Genetics Consortium: Loss of Heterozygosity at 11.q22.23 and survival in breast cancer: Results of a large European study. Genes, Chromosomes and Cancer, 25:212-221,1999.

10. Garcia-Closas, M., Egan, KM., Abruzzo, J., Newcomb, PA., Titus-Ernstoff, L., Franklin, T., Bender, PK., Beck, J.C., Le Marchand, L., Lum, A., Alavanja, M., Hayes, R.B., Rutter, J., Buetow, K., Brinton, L.A. and Rothman, N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiology, Biomarkers & Prevention. 10: 687-96, 2001

11. Brinckerhoff, C.E., Rutter, J.L. and Benbow, U. Interstitial collagenases as markers of tumor progression. Clin. Cancer Res. 6: 4823-4830, 2000.

12. Benbow, U., Tower, G.B., Wyatt, C.A., Buttice, G. and Brinckerhoff, C.E. High levels of MMP-1 expression in the absence of the 2G nucleotide polymorphism is mediated by p38 and ERK1/2 Mitogen-Activated Protein Kinases in VMM5 melanoma cells. J. Cellular Biochemistry. 86:307-319, 2002.

13. Ma, X.-J., Salunga, R., Tuggle, J.T., Gaudet, J.J., Enright, E., McQuary, P., Payette, T., Piston, M., Steckler, K., Zhang, B.M., Zhou, Y.-X., Varnolt, H., Smith, B., Gadd, M., Chatfield, E., Kessler, J., Baer, T.M., Erlander, M.G. and Sgroi, D.C. Gene expression profiles of human breast cancer progression. PNAS 100: 5974-5979, 2003.

APPENDIX COVER SHEET

Appendix
MMP-1 expression and patient #

Px	Dx	Genotype	Sample	Avg. Ct	St. Dev.	Avg. ng	St.Dev. ng
5	N	Unobtainable	5N	40.00	0.00	0.00	0.00
5	DCIS	Unobtainable	5DCIS	32.65	0.14	0.12	0.01
5	IDC	Unobtainable	5IDC	40.00	0.00	0.00	0.00
8	DCIS		8 DCIS	40.00	0.00	0.00	0.00
8	IDC		8 IDC	40.00	0.00	0.00	0.00
11	N	1G/2G	11N	35.65	0.22	0.01	0.00
11	DCIS	1G/2G	11DCIS	39.80	0.35	0.00	0.00
12	N		12N	39.75	0.43	0.00	0.00
12	DCIS		12DCIS	40.00	0.00	0.00	0.00
14	N		14N	37.31	0.40	0.00	0.00
14	DCIS		14DCIS	33.59	0.14	0.06	0.01
14	IDC		14IDC	32.67	0.09	0.12	0.01
22	ADH		22 ADH	40.00	0.00	0.00	0.00
22	DCIS		22 DCIS	39.97	0.06	0.00	0.00
25	DCIS		25 DCIS	40.00	0.00	0.00	0.00
25	IDC		25 IDC	39.54	0.80	0.00	0.00
30	N		30N	40.00	0.00	0.00	0.00
30	DCIS		30DCIS	40.00	0.00	0.00	0.00
30	IDC		30IDC	25.81	0.05	9.99	0.32
40	N		40 N	39.56	0.76	0.00	0.00
40	LCIS		40 LCIS	40.00	0.00	0.00	0.00
41	N	1G/2G	41N	40.00	0.00	0.00	0.00
41	DCIS	1G/2G	41DCIS	32.29	0.07	0.15	0.01
41	IDC	1G/2G	41IDC	32.93	0.21	0.10	0.01
43	N	1G/2G	43N	40.00	0.00	0.00	0.00
43	DCIS	1G/2G	43DCIS	31.46	0.05	0.26	0.01
43	IDC	1G/2G	43IDC	29.81	0.04	0.74	0.02
44	N	1G/1G	44N	37.18	0.29	0.01	0.00
44	DCIS	1G/1G	44DCIS	36.89	0.52	0.01	0.00
44	IDC	1G/1G	44IDC	29.89	0.11	0.71	0.05
45	N	1G/2G	45N	33.30	0.31	0.04	0.01
45	DCIS	1G/2G	45DCIS	30.88	0.14	0.19	0.02
57	N		57N	37.06	0.26	0.01	0.00
57	ADH		57ADH	34.02	0.12	0.05	0.00
57	DCIS		57DCIS	31.90	0.12	0.19	0.02
60	N	2G/2G	60N	39.93	0.12	0.00	0.00
60	DCIS	2G/2G	60DCIS	34.41	0.54	0.02	0.00
65	N	Unobtainable	65N	34.86	0.03	0.03	0.00
65	DCIS	Unobtainable	65DCIS	33.88	0.25	0.05	0.01
65	IDC	Unobtainable	65IDC	40.00	0.00	0.00	0.00
72	N	1G/2G	72N	32.56	0.02	0.12	0.00
72	DCIS	1G/2G	72DCIS	33.02	0.06	0.09	0.00
75	N	1G/2G	75N	40.00	0.00	0.00	0.00
75	DCIS	1G/2G	75DCIS	35.71	0.22	0.02	0.00
79	N	1G/1G	79 N	33.44	0.20	0.04	0.00
79	ADH	1G/1G	79 ADH	39.68	0.56	0.00	0.00
79	DCIS	1G/1G	79 DCIS	39.70	0.52	0.00	0.00
79	IDC	1G/1G	79 IDC	32.99	0.32	0.05	0.01
88	N	1G/2G	88 N	40.00	0.00	0.00	0.00
88	DCIS	1G/2G	88 DCIS	24.33	0.06	12.45	0.47
88	IDC	1G/2G	88 IDC	22.21	0.06	48.84	2.01

Appendix
MMP-1 expression and patient #

89	N	1G/2G	89 N	40.00	0.00	0.00	0.00
89	DCIS	1G/2G	89 DCIS	33.40	0.11	0.04	0.00
96	N	1G/2G	96 N	39.44	0.50	0.00	0.00
96	DCIS	1G/2G	96 DCIS	40.00	0.00	0.00	0.00
96	IDC	1G/2G	96 IDC	37.49	0.29	0.00	0.00
97	DCIS		97 DCIS	22.19	0.02	54.46	0.62
102	N	1G/1G	102 N	40.00	0.00	0.00	0.00
102	DCIS	1G/1G	102 DCIS	31.98	0.23	0.09	0.01
102	IDC	1G/1G	102 IDC	33.59	0.39	0.03	0.01
112	N	1G/1G	112 N	40.00	0.00	0.00	0.00
112	DCIS	1G/1G	112 DCIS	39.75	0.43	0.00	0.00
112	IDC	1G/1G	112 IDC	28.16	0.06	1.06	0.04
121	N	2G/2G	121 N	40.00	0.00	0.00	0.00
121	DCIS	2G/2G	121 DCIS	38.40	0.32	0.00	0.00
121	IDC	2G/2G	121 IDC	27.79	0.11	1.35	0.09
122	N	1G/2G	122 N	33.56	0.32	0.03	0.01
122	DCIS	1G/2G	122 DCIS	39.82	0.31	0.00	0.00
122	IDC	1G/2G	122 IDC	40.00	0.00	0.00	0.00
130	N	1G/2G	130N	40.00	0.00	0.00	0.00
130	DCIS	1G/2G	130DCIS	40.00	0.00	0.00	0.00
130	IDC	1G/2G	130IDC	40.00	0.00	0.00	0.00
131	N	2G/2G	131N	40.00	0.00	0.00	0.00
131	ADH	2G/2G	131ADH	33.96	0.20	0.02	0.00
131	DCIS	2G/2G	131DCIS	40.00	0.00	0.00	0.00
131	IDC	2G/2G	131IDC	32.15	0.08	0.08	0.00
133	N	1G/1G	133N	38.57	0.73	0.00	0.00
133	DCIS	1G/1G	133DCIS	24.35	0.16	12.68	1.33
133	IDC	1G/1G	133IDC	28.06	0.05	1.13	0.03
148	N	1G/2G	148N	35.33	0.29	0.01	0.00
148	DCIS	1G/2G	148DCIS	40.00	0.00	0.00	0.00
148	IDC	1G/2G	148IDC	32.66	0.24	0.06	0.01
152	N	1G/2G	152N	40.00	0.00	0.00	0.00
152	DCIS	1G/2G	152DCIS	28.93	0.12	0.65	0.05
153	N	1G/2G	153N	31.28	0.13	0.13	0.01
153	IDC	1G/2G	153IDC	40.00	0.00	0.00	0.00
169	N	1G/1G	169N	40.00	0.00	0.00	0.00
169	DCIS	1G/1G	169DCIS	27.20	0.06	1.98	0.08
169	IDC	1G/1G	169IDC	25.38	0.11	6.47	0.47
170	N	1G/2G	170N	37.56	0.10	0.00	0.00
170	DCIS	1G/2G	170DCIS	39.66	0.58	0.00	0.00
170	IDC	1G/2G	170IDC	27.13	0.06	2.24	0.10
173	N	1G/2G	173N	40.00	0.00	0.00	0.00
173	DCIS	1G/2G	173DCIS	40.00	0.00	0.00	0.00
173	IDC	1G/2G	173IDC	39.27	0.46	0.00	0.00
178	N	1G/1G	178N	40.00	0.00	0.00	0.00
178	DCIS	1G/1G	178DCIS	39.92	0.14	0.00	0.00
178	IDC	1G/1G	178IDC	27.86	0.17	1.40	0.15
179	N		179N	39.75	0.43	0.00	0.00
179	DCIS		179DCIS	40.00	0.00	0.00	0.00
179	IDC		179IDC	40.00	0.00	0.00	0.00
180	N	1G/2G	180N	35.88	0.39	0.01	0.00
180	ADH	1G/2G	180ADH	31.53	0.08	0.12	0.01
180	DCIS	1G/2G	180DCIS	31.43	0.18	0.13	0.01

Appendix
MMP-1 expression and patient #

180	IDC	1G/2G	180IDC	40.00	0.00	0.00	0.00
183	N	Unobtainable	183N	40.00	0.00	0.00	0.00
183	DCIS	Unobtainable	183DCIS	40.00	0.00	0.00	0.00
184	N	Unobtainable	184N	40.00	0.00	0.00	0.00
184	DCIS	Unobtainable	184DCIS	40.00	0.00	0.00	0.00
191	N	1G/1G	191N	32.13	0.06	0.09	0.00
191	ADH	1G/1G	191ADH	33.23	0.24	0.05	0.01
191	DCIS	1G/1G	191DCIS	29.31	0.11	0.55	0.04
193	N	1G/1G	193N	39.71	0.50	0.00	0.00
193	ADH	1G/1G	193ADH	37.27	0.41	0.00	0.00
193	DCIS	1G/1G	193DCIS	28.77	0.07	0.78	0.03
193	IDC	1G/1G	193IDC	29.33	0.10	0.55	0.03
198	N		198N	33.24	0.05	0.05	0.00
198	DCIS		198DCIS	37.57	0.42	0.00	0.00
198	IDC		198IDC	29.97	0.13	0.37	0.03
210	N		210 N	38.73	0.60	0.00	0.00
210	ADH		210 ADH	29.49	0.08	0.50	0.02
210	DCIS		210 DCIS	38.68	0.98	0.00	0.00
213	N		213N	36.64	0.33	0.01	0.00
213	ADH		213ADH	40.00	0.00	0.00	0.00
215	MPR		215 MPR	33.20	0.12	0.05	0.00
78-1	MPR		78-1 MPR	40.00	0.00	0.00	0.00
78-3	MPR		78-3 MPR	40.00	0.00	0.00	0.00
	1		Stock 1:4	20.17	0.02	190.83	2.27
	4		Stock 1:16	22.12	0.08	53.97	2.65
	7		Stock 1:64	24.38	0.06	12.38	0.51
	10		Stock 1:256	26.53	0.03	3.07	0.06
	1		Stock 1:4	21.25	0.04	194.97	5.54
	4		Stock 1:16	23.30	0.01	51.17	0.42
	7		Stock 1:64	25.42	0.03	12.89	0.22
	10		Stock 1:256	27.64	0.04	3.04	0.07
	1		Stock 1:4	20.06	0.08	194.88	9.72
	4		Stock 1:16	22.12	0.07	51.73	2.51
	7		Stock 1:64	24.30	0.09	12.69	0.74
	10		Stock 1:256	26.51	0.12	3.07	0.24
	1		Stock 1:4	20.10	0.05	191.63	5.63
	4		Stock 1:16	22.16	0.11	51.86	3.75
	7		Stock 1:64	24.31	0.17	13.31	1.36
	10		Stock 1:256	26.67	0.02	2.97	0.04
	1		Stock 1:4	20.21	0.08	195.06	10.46
	4		Stock 1:16	22.29	0.06	51.21	1.94
	7		Stock 1:64	24.44	0.08	12.90	0.61
	10		Stock 1:256	26.69	0.13	3.04	0.25
	10		NTC	40.00	0.00	0.00	0.00
	1		MCF-7 Unamp	37.73	0.74	0.00	0.00
	4		Daudi Unamp	35.52	0.13	0.01	0.00
	7		MCF-7 Amp	35.66	0.25	0.01	0.00
	10		Daudi Amp	33.03	0.22	0.05	0.01

5 Unobtainable
11 1G/2G
39 2G/2G
41 1G/2G

43	1G/2G
44	1G/1G
45	1G/2G
53	1G/2G
60	2G/2G
65	Unobtainable
72	1G/2G
75	1G/2G
79	1G/1G
88	1G/2G
89	1G/2G
96	1G/2G
102	1G/1G
112	1G/1G
121	2G/2G
122	1G/2G
130	1G/2G
131	2G/2G
133	1G/1G
148	1G/2G
152	1G/2G
153	1G/2G
169	1G/1G
170	1G/2G
173	1G/2G
178	1G/1G
180	1G/2G
183	Unobtainable
184	Unobtainable
191	1G/1G
193	1G/1G

Appendix
MMP-1 expression and tumor stage

Brinckerhoff, Constance E.

Px	Dx	Genotype	Sample	Avg. Ct	St. Dev.	Avg. ng	St.Dev. ng
5	N	Unobtainable	5N	40.00	0.00	0.00	0.00
11	N	1G/2G	11N	35.65	0.22	0.01	0.00
12	N		12N	39.75	0.43	0.00	0.00
14	N		14N	37.31	0.40	0.00	0.00
30	N		30N	40.00	0.00	0.00	0.00
40	N		40 N	39.56	0.76	0.00	0.00
41	N	1G/2G	41N	40.00	0.00	0.00	0.00
43	N	1G/2G	43N	40.00	0.00	0.00	0.00
44	N	1G/1G	44N	37.18	0.29	0.01	0.00
45	N	1G/2G	45N	33.30	0.31	0.04	0.01
57	N		57N	37.06	0.26	0.01	0.00
60	N	2G/2G	60N	39.93	0.12	0.00	0.00
65	N	Unobtainable	65N	34.86	0.03	0.03	0.00
72	N	1G/2G	72N	32.56	0.02	0.12	0.00
75	N	1G/2G	75N	40.00	0.00	0.00	0.00
79	N	1G/1G	79 N	33.44	0.20	0.04	0.00
88	N	1G/2G	88 N	40.00	0.00	0.00	0.00
89	N	1G/2G	89 N	40.00	0.00	0.00	0.00
96	N	1G/2G	96 N	39.44	0.50	0.00	0.00
102	N	1G/1G	102 N	40.00	0.00	0.00	0.00
112	N	1G/1G	112 N	40.00	0.00	0.00	0.00
121	N	2G/2G	121 N	40.00	0.00	0.00	0.00
122	N	1G/2G	122 N	33.56	0.32	0.03	0.01
130	N	1G/2G	130N	40.00	0.00	0.00	0.00
131	N	2G/2G	131N	40.00	0.00	0.00	0.00
133	N	1G/1G	133N	38.57	0.73	0.00	0.00
148	N	1G/2G	148N	35.33	0.29	0.01	0.00
152	N	1G/2G	152N	40.00	0.00	0.00	0.00
153	N	1G/2G	153N	31.28	0.13	0.13	0.01
169	N	1G/1G	169N	40.00	0.00	0.00	0.00
170	N	1G/2G	170N	37.56	0.10	0.00	0.00
173	N	1G/2G	173N	40.00	0.00	0.00	0.00
178	N	1G/1G	178N	40.00	0.00	0.00	0.00
179	N		179N	39.75	0.43	0.00	0.00
180	N	1G/2G	180N	35.88	0.39	0.01	0.00
183	N	Unobtainable	183N	40.00	0.00	0.00	0.00
184	N	Unobtainable	184N	40.00	0.00	0.00	0.00
191	N	1G/1G	191N	32.13	0.06	0.09	0.00
193	N	1G/1G	193N	39.71	0.50	0.00	0.00
198	N		198N	33.24	0.05	0.05	0.00
210	N		210 N	38.73	0.60	0.00	0.00
213	N		213N	36.64	0.33	0.01	0.00
22	ADH		22 ADH	40.00	0.00	0.00	0.00
57	ADH		57ADH	34.02	0.12	0.05	0.00
79	ADH	1G/1G	79 ADH	39.68	0.56	0.00	0.00
131	ADH	2G/2G	131ADH	33.96	0.20	0.02	0.00
180	ADH	1G/2G	180ADH	31.53	0.08	0.12	0.01
191	ADH	1G/1G	191ADH	33.23	0.24	0.05	0.01
193	ADH	1G/1G	193ADH	37.27	0.41	0.00	0.00
210	ADH		210 ADH	29.49	0.08	0.50	0.02
213	ADH		213ADH	40.00	0.00	0.00	0.00

Appendix
MMP-1 expression and tumor stage

5	DCIS	Unobtainable	5DCIS	32.65	0.14	0.12	0.01
8	DCIS		8 DCIS	40.00	0.00	0.00	0.00
11	DCIS	1G/2G	11DCIS	39.80	0.35	0.00	0.00
12	DCIS		12DCIS	40.00	0.00	0.00	0.00
14	DCIS		14DCIS	33.59	0.14	0.06	0.01
22	DCIS		22 DCIS	39.97	0.06	0.00	0.00
25	DCIS		25 DCIS	40.00	0.00	0.00	0.00
30	DCIS		30DCIS	40.00	0.00	0.00	0.00
41	DCIS	1G/2G	41DCIS	32.29	0.07	0.15	0.01
43	DCIS	1G/2G	43DCIS	31.46	0.05	0.26	0.01
44	DCIS	1G/1G	44DCIS	36.89	0.52	0.01	0.00
45	DCIS	1G/2G	45DCIS	30.88	0.14	0.19	0.02
57	DCIS		57DCIS	31.90	0.12	0.19	0.02
60	DCIS	2G/2G	60DCIS	34.41	0.54	0.02	0.00
65	DCIS	Unobtainable	65DCIS	33.88	0.25	0.05	0.01
72	DCIS	1G/2G	72DCIS	33.02	0.06	0.09	0.00
75	DCIS	1G/2G	75DCIS	35.71	0.22	0.02	0.00
79	DCIS	1G/1G	79 DCIS	39.70	0.52	0.00	0.00
88	DCIS	1G/2G	88 DCIS	24.33	0.06	12.45	0.47
89	DCIS	1G/2G	89 DCIS	33.40	0.11	0.04	0.00
96	DCIS	1G/2G	96 DCIS	40.00	0.00	0.00	0.00
97	DCIS		97 DCIS	22.19	0.02	54.46	0.62
102	DCIS	1G/1G	102 DCIS	31.98	0.23	0.09	0.01
112	DCIS	1G/1G	112 DCIS	39.75	0.43	0.00	0.00
121	DCIS	2G/2G	121 DCIS	38.40	0.32	0.00	0.00
122	DCIS	1G/2G	122 DCIS	39.82	0.31	0.00	0.00
130	DCIS	1G/2G	130DCIS	40.00	0.00	0.00	0.00
131	DCIS	2G/2G	131DCIS	40.00	0.00	0.00	0.00
133	DCIS	1G/1G	133DCIS	24.35	0.16	12.68	1.33
148	DCIS	1G/2G	148DCIS	40.00	0.00	0.00	0.00
152	DCIS	1G/2G	152DCIS	28.93	0.12	0.65	0.05
169	DCIS	1G/1G	169DCIS	27.20	0.06	1.98	0.08
170	DCIS	1G/2G	170DCIS	39.66	0.58	0.00	0.00
173	DCIS	1G/2G	173DCIS	40.00	0.00	0.00	0.00
178	DCIS	1G/1G	178DCIS	39.92	0.14	0.00	0.00
179	DCIS		179DCIS	40.00	0.00	0.00	0.00
180	DCIS	1G/2G	180DCIS	31.43	0.18	0.13	0.01
183	DCIS	Unobtainable	183DCIS	40.00	0.00	0.00	0.00
184	DCIS	Unobtainable	184DCIS	40.00	0.00	0.00	0.00
191	DCIS	1G/1G	191DCIS	29.31	0.11	0.55	0.04
193	DCIS	1G/1G	193DCIS	28.77	0.07	0.78	0.03
198	DCIS		198DCIS	37.57	0.42	0.00	0.00
210	DCIS		210 DCIS	38.68	0.98	0.00	0.00
5	IDC	Unobtainable	5IDC	40.00	0.00	0.00	0.00
8	IDC		8 IDC	40.00	0.00	0.00	0.00
14	IDC		14IDC	32.67	0.09	0.12	0.01
25	IDC		25 IDC	39.54	0.80	0.00	0.00
30	IDC		30IDC	25.81	0.05	9.99	0.32
41	IDC	1G/2G	41IDC	32.93	0.21	0.10	0.01
43	IDC	1G/2G	43IDC	29.81	0.04	0.74	0.02
44	IDC	1G/1G	44IDC	29.89	0.11	0.71	0.05
65	IDC	Unobtainable	65IDC	40.00	0.00	0.00	0.00
79	IDC	1G/1G	79 IDC	32.99	0.32	0.05	0.01

Appendix
MMP-1 expression and tumor stage

88	IDC	1G/2G	88 IDC	22.21	0.06	48.84	2.01
96	IDC	1G/2G	96 IDC	37.49	0.29	0.00	0.00
102	IDC	1G/1G	102 IDC	33.59	0.39	0.03	0.01
112	IDC	1G/1G	112 IDC	28.16	0.06	1.06	0.04
121	IDC	2G/2G	121 IDC	27.79	0.11	1.35	0.09
122	IDC	1G/2G	122 IDC	40.00	0.00	0.00	0.00
130	IDC	1G/2G	130IDC	40.00	0.00	0.00	0.00
131	IDC	2G/2G	131IDC	32.15	0.08	0.08	0.00
133	IDC	1G/1G	133IDC	28.06	0.05	1.13	0.03
148	IDC	1G/2G	148IDC	32.66	0.24	0.06	0.01
153	IDC	1G/2G	153IDC	40.00	0.00	0.00	0.00
169	IDC	1G/1G	169IDC	25.38	0.11	6.47	0.47
170	IDC	1G/2G	170IDC	27.13	0.06	2.24	0.10
173	IDC	1G/2G	173IDC	39.27	0.46	0.00	0.00
178	IDC	1G/1G	178IDC	27.86	0.17	1.40	0.15
179	IDC		179IDC	40.00	0.00	0.00	0.00
180	IDC	1G/2G	180IDC	40.00	0.00	0.00	0.00
193	IDC	1G/1G	193IDC	29.33	0.10	0.55	0.03
198	IDC		198IDC	29.97	0.13	0.37	0.03
40	LCIS		40 LCIS	40.00	0.00	0.00	0.00
215	MPR		215 MPR	33.20	0.12	0.05	0.00
78-1	MPR		78-1 MPR	40.00	0.00	0.00	0.00
78-3	MPR		78-3 MPR	40.00	0.00	0.00	0.00
	1		Stock 1:4	20.17	0.02	190.83	2.27
	1		Stock 1:4	21.25	0.04	194.97	5.54
	1		Stock 1:4	20.06	0.08	194.88	9.72
	1		Stock 1:4	20.10	0.05	191.63	5.63
	1		Stock 1:4	20.21	0.08	195.06	10.46
	1		MCF-7 Unamp	37.73	0.74	0.00	0.00
	4		Stock 1:16	22.12	0.08	53.97	2.65
	4		Stock 1:16	23.30	0.01	51.17	0.42
	4		Stock 1:16	22.12	0.07	51.73	2.51
	4		Stock 1:16	22.16	0.11	51.86	3.75
	4		Stock 1:16	22.29	0.06	51.21	1.94
	4		Daudi Unamp	35.52	0.13	0.01	0.00
	7		Stock 1:64	24.38	0.06	12.38	0.51
	7		Stock 1:64	25.42	0.03	12.89	0.22
	7		Stock 1:64	24.30	0.09	12.69	0.74
	7		Stock 1:64	24.31	0.17	13.31	1.36
	7		Stock 1:64	24.44	0.08	12.90	0.61
	7		MCF-7 Amp	35.66	0.25	0.01	0.00
	10		Stock 1:256	26.53	0.03	3.07	0.06
	10		Stock 1:256	27.64	0.04	3.04	0.07
	10		Stock 1:256	26.51	0.12	3.07	0.24
	10		Stock 1:256	26.67	0.02	2.97	0.04
	10		Stock 1:256	26.69	0.13	3.04	0.25
	10		NTC	40.00	0.00	0.00	0.00
	10		Daudi Amp	33.03	0.22	0.05	0.01

Appendix
MMP-1 expression and genotype

Brinckerhoff, Constance E.

Px	Dx	Genotype	Sample	Avg. Ct	St. Dev.	Avg. ng	St.Dev. ng
44	N	1G/1G	44N	37.18	0.29	0.01	0.00
44	DCIS	1G/1G	44DCIS	36.89	0.52	0.01	0.00
44	IDC	1G/1G	44IDC	29.89	0.11	0.71	0.05
79	N	1G/1G	79 N	33.44	0.20	0.04	0.00
79	ADH	1G/1G	79 ADH	39.68	0.56	0.00	0.00
79	DCIS	1G/1G	79 DCIS	39.70	0.52	0.00	0.00
79	IDC	1G/1G	79 IDC	32.99	0.32	0.05	0.01
102	N	1G/1G	102 N	40.00	0.00	0.00	0.00
102	DCIS	1G/1G	102 DCIS	31.98	0.23	0.09	0.01
102	IDC	1G/1G	102 IDC	33.59	0.39	0.03	0.01
112	N	1G/1G	112 N	40.00	0.00	0.00	0.00
112	DCIS	1G/1G	112 DCIS	39.75	0.43	0.00	0.00
112	IDC	1G/1G	112 IDC	28.16	0.06	1.06	0.04
133	N	1G/1G	133N	38.57	0.73	0.00	0.00
133	DCIS	1G/1G	133DCIS	24.35	0.16	12.68	1.33
133	IDC	1G/1G	133IDC	28.06	0.05	1.13	0.03
169	N	1G/1G	169N	40.00	0.00	0.00	0.00
169	DCIS	1G/1G	169DCIS	27.20	0.06	1.98	0.08
169	IDC	1G/1G	169IDC	25.38	0.11	6.47	0.47
178	N	1G/1G	178N	40.00	0.00	0.00	0.00
178	DCIS	1G/1G	178DCIS	39.92	0.14	0.00	0.00
178	IDC	1G/1G	178IDC	27.86	0.17	1.40	0.15
191	N	1G/1G	191N	32.13	0.06	0.09	0.00
191	ADH	1G/1G	191ADH	33.23	0.24	0.05	0.01
191	DCIS	1G/1G	191DCIS	29.31	0.11	0.55	0.04
193	N	1G/1G	193N	39.71	0.50	0.00	0.00
193	ADH	1G/1G	193ADH	37.27	0.41	0.00	0.00
193	DCIS	1G/1G	193DCIS	28.77	0.07	0.78	0.03
193	IDC	1G/1G	193IDC	29.33	0.10	0.55	0.03
11	N	1G/2G	11N	35.65	0.22	0.01	0.00
11	DCIS	1G/2G	11DCIS	39.80	0.35	0.00	0.00
41	N	1G/2G	41N	40.00	0.00	0.00	0.00
41	DCIS	1G/2G	41DCIS	32.29	0.07	0.15	0.01
41	IDC	1G/2G	41IDC	32.93	0.21	0.10	0.01
43	N	1G/2G	43N	40.00	0.00	0.00	0.00
43	DCIS	1G/2G	43DCIS	31.46	0.05	0.26	0.01
43	IDC	1G/2G	43IDC	29.81	0.04	0.74	0.02
45	N	1G/2G	45N	33.30	0.31	0.04	0.01
45	DCIS	1G/2G	45DCIS	30.88	0.14	0.19	0.02
72	N	1G/2G	72N	32.56	0.02	0.12	0.00
72	DCIS	1G/2G	72DCIS	33.02	0.06	0.09	0.00
75	N	1G/2G	75N	40.00	0.00	0.00	0.00
75	DCIS	1G/2G	75DCIS	35.71	0.22	0.02	0.00
88	N	1G/2G	88 N	40.00	0.00	0.00	0.00
88	DCIS	1G/2G	88 DCIS	24.33	0.06	12.45	0.47
88	IDC	1G/2G	88 IDC	22.21	0.06	48.84	2.01
89	N	1G/2G	89 N	40.00	0.00	0.00	0.00
89	DCIS	1G/2G	89 DCIS	33.40	0.11	0.04	0.00
96	N	1G/2G	96 N	39.44	0.50	0.00	0.00
96	DCIS	1G/2G	96 DCIS	40.00	0.00	0.00	0.00
96	IDC	1G/2G	96 IDC	37.49	0.29	0.00	0.00
122	N	1G/2G	122 N	33.56	0.32	0.03	0.01

122	DCIS	1G/2G	122 DCIS	39.82	0.31	0.00	0.00
122	IDC	1G/2G	122 IDC	40.00	0.00	0.00	0.00
130	N	1G/2G	130N	40.00	0.00	0.00	0.00
130	DCIS	1G/2G	130DCIS	40.00	0.00	0.00	0.00
130	IDC	1G/2G	130IDC	40.00	0.00	0.00	0.00
148	N	1G/2G	148N	35.33	0.29	0.01	0.00
148	DCIS	1G/2G	148DCIS	40.00	0.00	0.00	0.00
148	IDC	1G/2G	148IDC	32.66	0.24	0.06	0.01
152	N	1G/2G	152N	40.00	0.00	0.00	0.00
152	DCIS	1G/2G	152DCIS	28.93	0.12	0.65	0.05
153	N	1G/2G	153N	31.28	0.13	0.13	0.01
153	IDC	1G/2G	153IDC	40.00	0.00	0.00	0.00
170	N	1G/2G	170N	37.56	0.10	0.00	0.00
170	DCIS	1G/2G	170DCIS	39.66	0.58	0.00	0.00
170	IDC	1G/2G	170IDC	27.13	0.06	2.24	0.10
173	N	1G/2G	173N	40.00	0.00	0.00	0.00
173	DCIS	1G/2G	173DCIS	40.00	0.00	0.00	0.00
173	IDC	1G/2G	173IDC	39.27	0.46	0.00	0.00
180	N	1G/2G	180N	35.88	0.39	0.01	0.00
180	ADH	1G/2G	180ADH	31.53	0.08	0.12	0.01
180	DCIS	1G/2G	180DCIS	31.43	0.18	0.13	0.01
180	IDC	1G/2G	180IDC	40.00	0.00	0.00	0.00
60	N	2G/2G	60N	39.93	0.12	0.00	0.00
60	DCIS	2G/2G	60DCIS	34.41	0.54	0.02	0.00
121	N	2G/2G	121 N	40.00	0.00	0.00	0.00
121	DCIS	2G/2G	121 DCIS	38.40	0.32	0.00	0.00
121	IDC	2G/2G	121 IDC	27.79	0.11	1.35	0.09
131	N	2G/2G	131N	40.00	0.00	0.00	0.00
131	ADH	2G/2G	131ADH	33.96	0.20	0.02	0.00
131	DCIS	2G/2G	131DCIS	40.00	0.00	0.00	0.00
131	IDC	2G/2G	131IDC	32.15	0.08	0.08	0.00
5	N	Unobtainable	5N	40.00	0.00	0.00	0.00
5	DCIS	Unobtainable	5DCIS	32.65	0.14	0.12	0.01
5	IDC	Unobtainable	5IDC	40.00	0.00	0.00	0.00
65	N	Unobtainable	65N	34.86	0.03	0.03	0.00
65	DCIS	Unobtainable	65DCIS	33.88	0.25	0.05	0.01
65	IDC	Unobtainable	65IDC	40.00	0.00	0.00	0.00
183	N	Unobtainable	183N	40.00	0.00	0.00	0.00
183	DCIS	Unobtainable	183DCIS	40.00	0.00	0.00	0.00
184	N	Unobtainable	184N	40.00	0.00	0.00	0.00
184	DCIS	Unobtainable	184DCIS	40.00	0.00	0.00	0.00
8	DCIS		8 DCIS	40.00	0.00	0.00	0.00
8	IDC		8 IDC	40.00	0.00	0.00	0.00
12	N		12N	39.75	0.43	0.00	0.00
12	DCIS		12DCIS	40.00	0.00	0.00	0.00
14	N		14N	37.31	0.40	0.00	0.00
14	DCIS		14DCIS	33.59	0.14	0.06	0.01
14	IDC		14IDC	32.67	0.09	0.12	0.01
22	ADH		22 ADH	40.00	0.00	0.00	0.00
22	DCIS		22 DCIS	39.97	0.06	0.00	0.00
25	DCIS		25 DCIS	40.00	0.00	0.00	0.00
25	IDC		25 IDC	39.54	0.80	0.00	0.00
30	N		30N	40.00	0.00	0.00	0.00

30	DCIS		30DCIS	40.00	0.00	0.00	0.00
30	IDC		30IDC	25.81	0.05	9.99	0.32
40	N		40 N	39.56	0.76	0.00	0.00
40	LCIS		40 LCIS	40.00	0.00	0.00	0.00
57	N		57N	37.06	0.26	0.01	0.00
57	ADH		57ADH	34.02	0.12	0.05	0.00
57	DCIS		57DCIS	31.90	0.12	0.19	0.02
97	DCIS		97 DCIS	22.19	0.02	54.46	0.62
179	N		179N	39.75	0.43	0.00	0.00
179	DCIS		179DCIS	40.00	0.00	0.00	0.00
179	IDC		179IDC	40.00	0.00	0.00	0.00
198	N		198N	33.24	0.05	0.05	0.00
198	DCIS		198DCIS	37.57	0.42	0.00	0.00
198	IDC		198IDC	29.97	0.13	0.37	0.03
210	N		210 N	38.73	0.60	0.00	0.00
210	ADH		210 ADH	29.49	0.08	0.50	0.02
210	DCIS		210 DCIS	38.68	0.98	0.00	0.00
213	N		213N	36.64	0.33	0.01	0.00
213	ADH		213ADH	40.00	0.00	0.00	0.00
215	MPR		215 MPR	33.20	0.12	0.05	0.00
78-1	MPR		78-1 MPR	40.00	0.00	0.00	0.00
78-3	MPR		78-3 MPR	40.00	0.00	0.00	0.00
	1		Stock 1:4	20.17	0.02	190.83	2.27
	4		Stock 1:1(	22.12	0.08	53.97	2.65
	7		Stock 1:6-	24.38	0.06	12.38	0.51
	10		Stock 1:25	26.53	0.03	3.07	0.06
	1		Stock 1:4	21.25	0.04	194.97	5.54
	4		Stock 1:1(	23.30	0.01	51.17	0.42
	7		Stock 1:6-	25.42	0.03	12.89	0.22
	10		Stock 1:25	27.64	0.04	3.04	0.07
	1		Stock 1:4	20.06	0.08	194.88	9.72
	4		Stock 1:1(	22.12	0.07	51.73	2.51
	7		Stock 1:6-	24.30	0.09	12.69	0.74
	10		Stock 1:25	26.51	0.12	3.07	0.24
	1		Stock 1:-	20.10	0.05	191.63	5.63
	4		Stock 1:1	22.16	0.11	51.86	3.75
	7		Stock 1:6	24.31	0.17	13.31	1.36
	10		Stock 1:2	26.67	0.02	2.97	0.04
	1		Stock 1:4	20.21	0.08	195.06	10.46
	4		Stock 1:1(	22.29	0.06	51.21	1.94
	7		Stock 1:6-	24.44	0.08	12.90	0.61
	10		Stock 1:25	26.69	0.13	3.04	0.25
	10		NTC	40.00	0.00	0.00	0.00
	1		MCF-7 Unam	37.73	0.74	0.00	0.00
	4		Daudi Unam	35.52	0.13	0.01	0.00
	7		MCF-7 Amp	35.66	0.25	0.01	0.00
	10		Daudi Amp	33.03	0.22	0.05	0.01